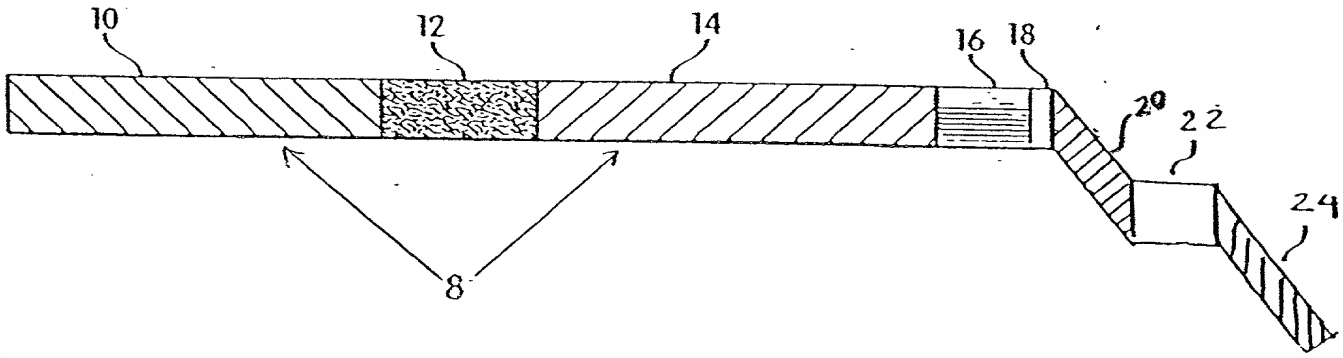
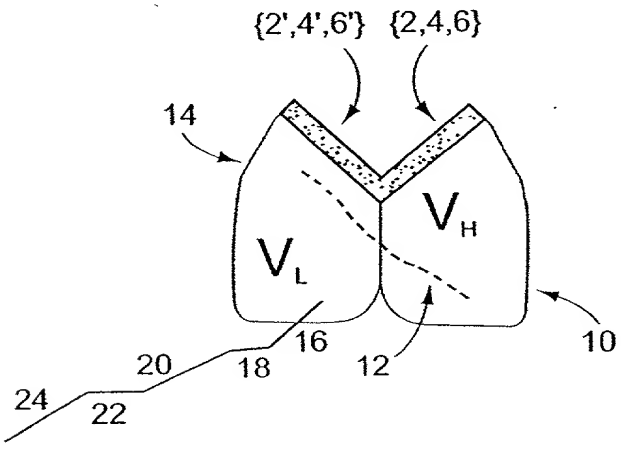
FIGURE 1

SINGLE CHAIN BINDING POLYPEPTIDE



(a) Extended Polypeptide



(b) Folded Protein

FIGURE 2

SINGLE CHAIN
BINDING POLYPEPTIDE SHOWING
LOCATIONS OF COMPLEMENTARITY
DETERMINING REGIONS, POLYPEPTIDE
SPACER REGIONS, AND EFFECTOR REGIONS

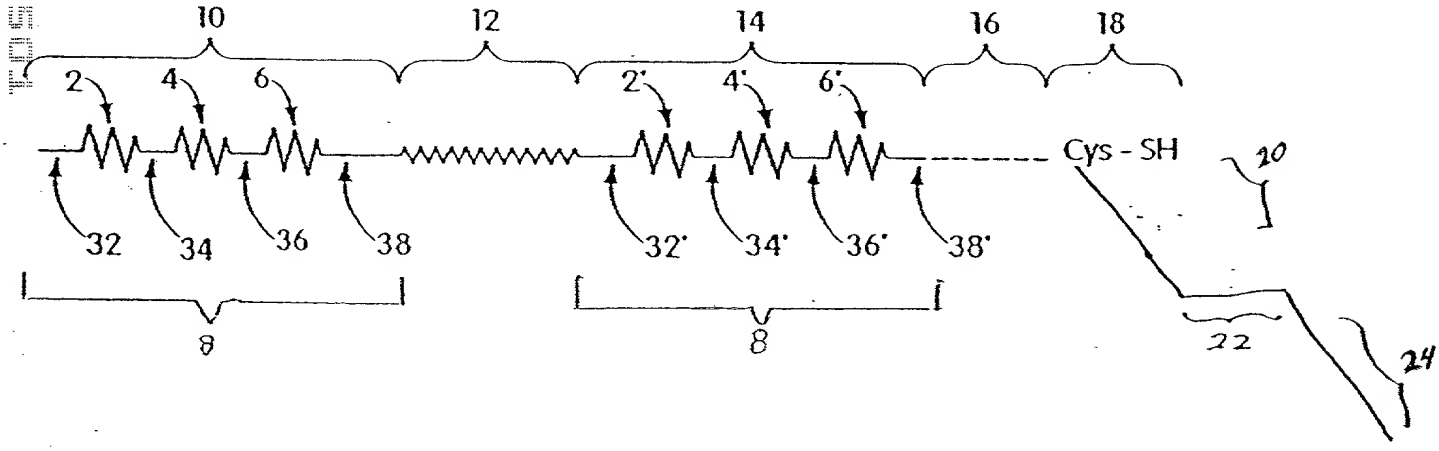


FIGURE 3

C6.5 sFv
AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLLQSGAE LKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYGHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCAAWDDSL
SGWVFGGGTK LTVLG

FIGURE 4

C6.5 sFv
NUCLEOTIDE SEQUENCE

5'caggtgcagctgttgcagtctggggcagagttgaaaaaacccggggagtctctgaagatctcctgtaagggttctggataca
gctttaccagctactggatcgcctgggtgcgccagatgcccgggaaaggcctggagtacatggggctcatctatcctggtgactc
tgacaccaaatacagcccgtccttccaaggccaggtcaccatctcagtcgacaagtccgtcagcactgcctacttgcaatggagc
agtctgaagcctcggacagcgccgtgtatttttgtgcgagacatgacgtgggatattgcagtagttccaactgcgcaaagtggcc
tgaatacttccagcattggggccagggcaccctggtcaccgtctcctcaggtggaggcggttcaggcggaggtggctctggcg
gtggcggatcgcagtctgttgacgcagccgccctcagtgtctgcggccccaggacagaaggtcaccatctcctgctctggaa
gcagctccaacattgggaataattatgtatcctggtaccagcagctcccaggaacagcccccaaactcctcatctatggtcacacca
atcggcccgcaggggtccctgaccgattctctggctccaagtctggcacctcagcctccctggccatcagtgggttccggtccga
ggatgaggctgattattactgtgcagcatgggatgacagcctgagtggttgggtgttcggcggagggaccaagctgaccgtcct
aggt 3'

FIGURE 5

C6ML3-9 sFv'
AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAHH HHHHGGGGC-

FIGURE 6

C6ML3-9 sFv'
NUCLEOTIDE SEQUENCE

5' caggtgcagctggtgcagtctggggcagaggtgaaaaagcccggggagtctctgaagatctcctgtaagggttctggata
cagctttaccagctactggatcgcctgggtgcgccagatgcccgggaaaggcctggagtacatggggctcatctatcctg
gtgactctgacaccaaatacagcccgtccttccaaggccaggtcaccatctcagtcgacaagtccgtcagcactgcctac
ttgcaatggagcagtctgaagccctcggacagcgccgtgtatttttgtgcgagacatgacgtgggatattgcagtagttc
caactgcgcaaagtggcctgaatacttccagcattggggccagggcaccctggtcaccgtctcctcaggtggaggcggtt
caggcggaggtggctctggcggtggcggatcgcagtctgtgttgacgcagccgccctcagtgtctgcggccccaggacag
aaggtcaccatctcctgctctggaagcagctccaacattgggaataattatgtatcctggtaccagcagctcccaggaac
agccccccaaactcctcatctatgatcacaccaatcggcccgcaggggtccctgaccgattctctggctccaagtctggca
cctcagcctccctggccatcagtgggttccggtccgaggatgaggctgattattactgtgcctcctgggactacaccctc
tcgggctgggtgttcggcggaggaaccaagctgaccgtcctaggtgcggccgcacaccatcatcaccatcacggtggtgg
cggctgc 3'

FIGURE 7

C6ML3-9sFv'-L1-KDEL
AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAHH HHHHGGGGCL ESSSSGSEKD EL-

FIGURE 8

C6ML3-9 sFv'-L1-KDEL
NUCLEOTIDE SEQUENCE

5' caggtgcagctggtgcagtctggggcagaggtgaaaaagcccggggagtctctgaagatctcctgtaagggttctggata
cagctttaccagctactggatcgcctgggtgcgccagatgcccgggaaaggcctggagtacatggggctcatctatcctg
gtgactctgacaccaaatacagcccgtccttccaaggccaggtcaccatctcagtcgacaagtccgtcagcactgcctac
ttgcaatggagcagtctgaagccctcggacagcgccgtgtatttttgtgcgagacatgacgtgggatattgcagtagttc
caactgcgcaaagtggcctgaatacttccagcattggggccagggcaccctggtcaccgtctcctcaggtggaggcggtt
caggcggaggtggctctggcggtggcggatcgcagtctgtgttgacgcagccgccctcagtgtctgcggccccaggacag
aaggtcaccatctcctgctctggaagcagctccaacattgggaataattatgtatcctggtaccagcagctcccaggaac
agcccccaaactcctcatctatgatcacaccaatcggcccgcaggggtccctgaccgattctctggctccaagtctggca
cctcagcctccctggccatcagtgggttccggtccgaggatgaggctgattattactgtgcctcctgggactacaccctc
tcgggctgggtgttcggcggaggaaccaagctgaccgtcctaggtgcggccgcacaccatcatcaccatcacggtggtgg
cggctgcctcgagtcct ctagctctgg atccgaaaaa gatgaactg3'

FIGURE 9

C6ML3-9 sFv' -L2-KDEL
AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAHH HHHHGGGGCL ESSSSGSSSS GSEKDEL-

FIGURE 10

C6ML3-9sFv'-L2-KDEL
NUCLEOTIDE SEQUENCE

5' caggtgcagctggtgcagtctggggcagaggtgaaaaagcccggggagtctctgaagatctcctgtaagggttctggata
cagctttaccagctactggatcgcctgggtgcgccagatgcccgggaaaggcctggagtacatggggctcatctatcctg
gtgactctgacaccaaatacagcccgtccttccaaggccaggtcaccatctcagtcgacaagtccgtcagcactgcctac
ttgcaatggagcagtctgaagccctcggacagcgccgtgtatttttgtgcgagacatgacgtgggatattgcagtagttc
caactgcgcaaagtggcctgaatacttccagcattggggccagggcaccctggtcaccgtctcctcaggtggaggcggtt
caggcggaggtggctctggcggtggcggatcgcagtctgtgttgacgcagccgccctcagtgtctgcggccccaggacag
aaggtcaccatctcctgctctggaagcagctccaacattgggaataattatgtatcctggtaccagcagctcccaggaac
agcccccaaactcctcatctatgatcacaccaatcggcccgcaggggtccctgaccgattctctggctccaagtctggca
cctcagcctccctggccatcagtgggttccggtccgaggatgaggctgattattactgtgcctcctgggactacaccctc
tcgggctgggtgttcggcggaggaaccaagctgaccgtcctaggtgcggccgcacaccatcatcaccatcacggtggtgg
cggctgcctcgagtcta gcagctccgg ttcctctagc tctggatccg aaaaagatga actg 3'

FIGURE 11

C6ML3-9 sFv'-L2-H14
AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAHH HHHHGGGGCL ESSSSGSSSS
GSKKSAKKTP KKAKKP-

FIGURE 12

C6ML3-9 sFv' -L2-H14
NUCLEOTIDE SEOUENCE

5' caggtgcagctggtgcagtctggggcagaggtgaaaaagcccggggagtctctgaagatctcctgtaagggttctggata
cagctttaccagctactggatcgcctgggtgcgccagatgcccgggaaaggcctggagtacatggggctcatctatcctg
gtgactctgacaccaaatacagcccgtccttccaaggccaggtcaccatctcagtcgacaagtccgtcagcactgcctac
ttgcaatggagcagtctgaagccctcggacagcgccgtgtatttttgtgcgagacatgacgtgggatattgcagtagttc
caactgcgcaaagtggcctgaatacttccagcattggggccagggcaccctggtcaccgtctcctcaggtggaggcggtt
caggcggaggtggctctggcggtggcggatcgcagtctgtgttgacgcagccgccctcagtgtctgcggccccaggacag
aaggtcaccatctcctgctctggaagcagctccaacattgggaataattatgtatcctggtaccagcagctcccaggaac
agcccccaaactcctcatctatgatcacaccaatcggcccgcaggggtccctgaccgattctctggctccaagtctggca
cctcagcctccctggccatcagtgggttccggtccgaggatgaggctgattattactgtgcctcctgggactacaccctc
tcgggctgggtgttcggcggaggaaccaagctgaccgtcctaggtgcggccgcacaccatcatcaccatcacggtggtgg
cggctgc ctcgagtcta gcagctccgg ttcctctagc tctggatcca agaaaagcgc gaaaaagacc ccgaagaaag
cgaagaaacc g 3'

FIGURE 13

C6ML3-9sFv'-L2-nls
AMINO ACID SEQUENCE

(N-terminus to C-terminus)

-QVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIAWVRQM PGKGLEYMGL
IYPGDSDTKY SPSFQGQVTI SVDKSVSTAY LQWSSLKPSD SAVYFCARHD
VGYCSSSNCA KWPEYFQHWG QGTLVTVSSG GGGSGGGGSG
GGGSQSVLTQ PPSVSAAPGQ KVTISCSGSS SNIGNNYVSW YQQLPGTAPK
LLIYDHTNRP AGVPDRFSGS KSGTSASLAI SGFRSEDEAD YYCASWDYTL
SGWVFGGGTK LTVLGAAAHH HHHHGGGGCL ESSSSGSSSS
GSTPPKKKRK V

FIGURE 14

C6ML3-9 sFv'-L2-nls
NUCLEOTIDE SEQUENCE

5' caggtgcagctggtgcagtctggggcagaggtgaaaaagcccggggagtctctgaagatctcctgtaagggttctggata
cagctttaccagctactggatcgcctgggtgcgccagatgcccgggaaaggcctggagtacatggggctcatctatcctg
gtgactctgacaccaaatacagcccgtccttccaaggccaggtcaccatctcagtcgacaagtccgtcagcactgcctac
ttgcaatggagcagtctgaagccctcggacagcgccgtgtatttttgtgcgagacatgacgtgggatattgcagtagttc
caactgcgcaaagtggcctgaatacttccagcattggggccagggcaccctggtcaccgtctcctcaggtggaggcggtt
caggcggaggtggctctggcggtggcggatcgcagtctgtgttgacgcagccgccctcagtgtctgcggccccaggacag
aaggtcaccatctcctgctctggaagcagctccaacattgggaataattatgtatcctggtaccagcagctcccaggaac
agcccccaaactcctcatctatgatcacaccaatcggcccgcaggggtccctgaccgattctctggctccaagtctggca
cctcagcctccctggccatcagtgggttccggtccgaggatgaggctgattattactgtgcctcctgggactacaccctc
tcgggctgggtgttcggcggaggaaccaagctgaccgtcctaggtgcggccgcacaccatcatcaccatcacggtggtgg
cggctgc ctcgagtcta gcagctccgg ttcctctagc tctggatcca ctccgccgaa aaagaaacgt aaagtg 3'

Figure 15. C6ML3-9 sFv' and its salmon protamine conjugate binds specifically to the erbB-2 positive ovarian cancer cells

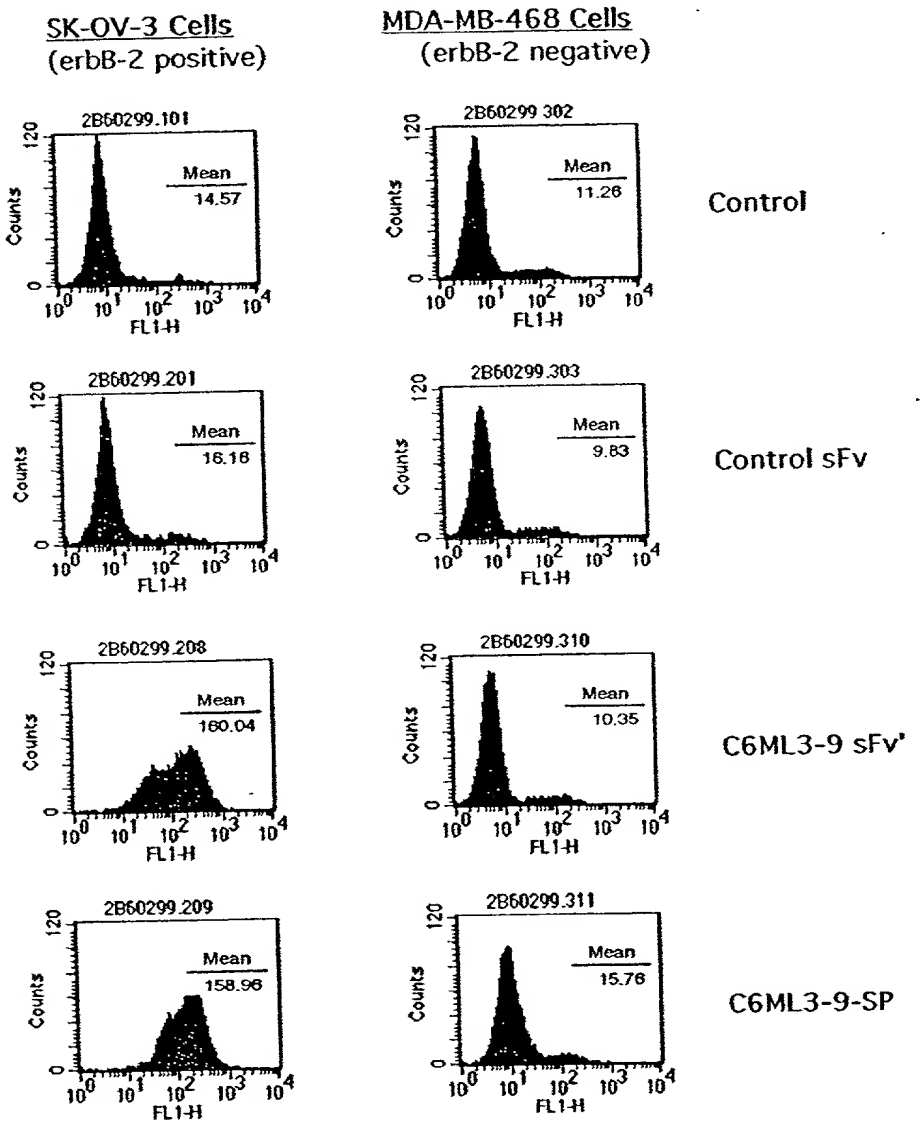


Figure 16. FACS Analysis of the erbB-2 Binding Activities of Bacterially Expressed C6ML3-9 sFv' and its Derivatives

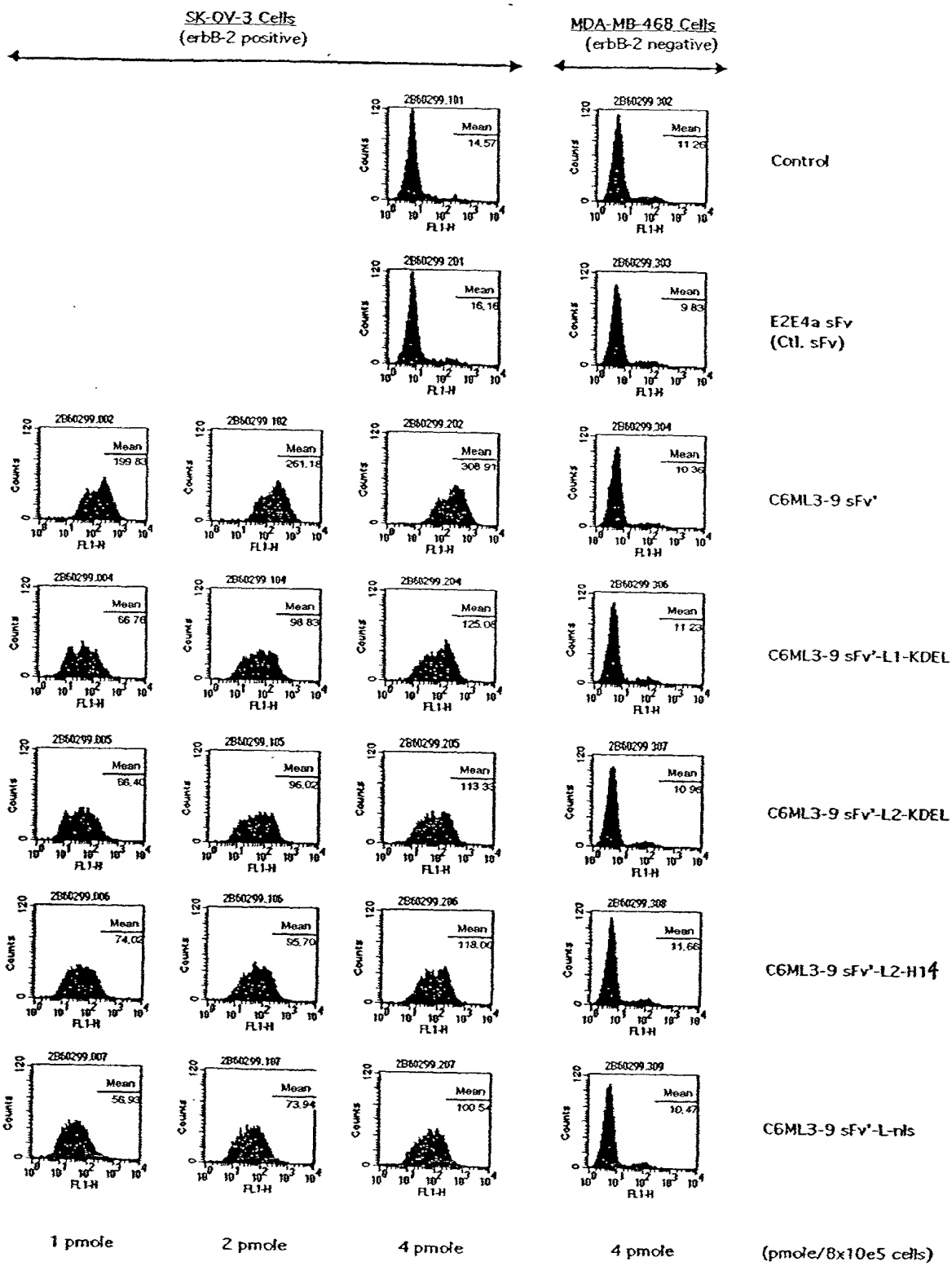
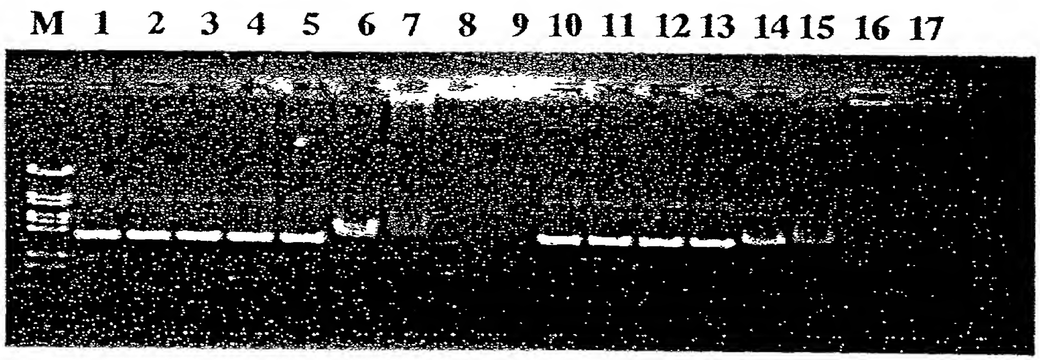


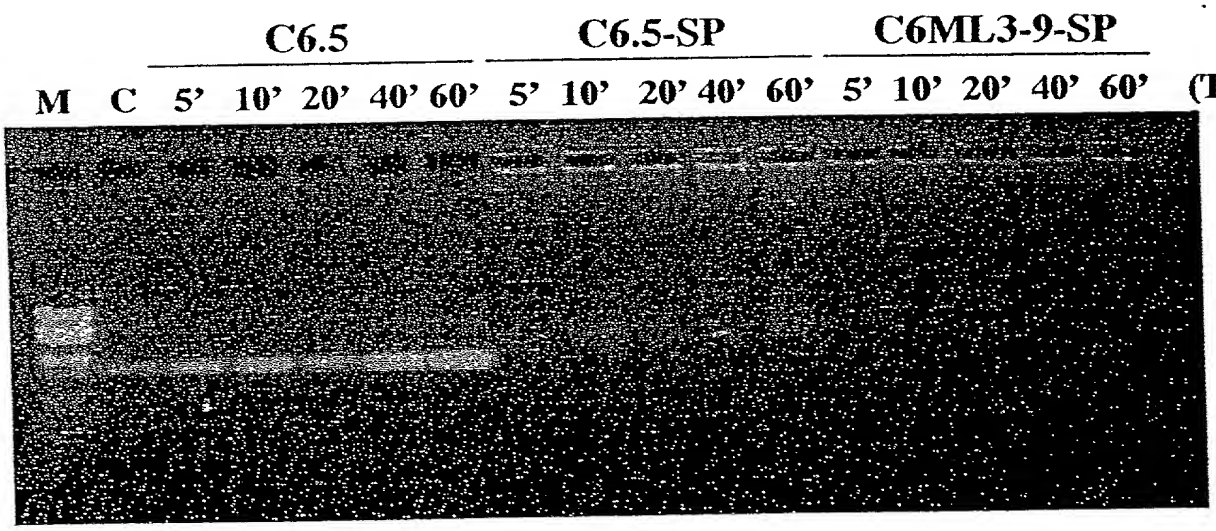
Figure 17. Gel Shift Analysis of the C6.5-SP-DNA and C6ML3-9-SP-DNA Complex



M. DNA marker - λ DNA BstEII digest
1. 200 ng pGL3 DNA
2. 200 ng pGL3 DNA + 1.45 pmol C6.5
3. 200 ng pGL3 DNA + 2.90 pmol C6.5
4. 200 ng pGL3 DNA + 5.80 pmol C6.5
5. 200 ng pGL3 DNA + 11.6 pmol C6.5
6. 200 ng pGL3 DNA + 1.45 pmol C6.5-SP
7. 200 ng pGL3 DNA + 2.90 pmol C6.5-SP
8. 200 ng pGL3 DNA + 5.80 pmol C6.5-SP
9. 200 ng pGL3 DNA + 11.6 pmol C6.5-SP
10. 200 ng pGL3 DNA + 1.45 pmol C6ML3-9
11. 200 ng pGL3 DNA + 2.90 pmol C6ML3-9
12. 200 ng pGL3 DNA + 5.80 pmol C6ML3-9
13. 200 ng pGL3 DNA + 11.6 pmol C6ML3-9
14. 200 ng pGL3 DNA + 1.45 pmol C6ML3-9-SP
15. 200 ng pGL3 DNA + 2.90 pmol C6ML3-9-SP
16. 200 ng pGL3 DNA + 5.80 pmol C6ML3-9-SP
17. 200 ng pGL3 DNA + 11.6 pmol C6ML3-9-SP

*0.8% agarose gel in 1xTAE, 150v, RT, ~1hr, EtBr staining overnight

Figure 18. Kinetic Study of the C6.5-SP-DNA and C6ML3-9-SP-DNA Complex Formation



M. DNA marker - λ DNA BstEII digest

C. · 200 ng pGL3 DNA alone

* The rest of the lanes - 200 ng pGL3 DNA incubated with 5.8 pmol proteins as indicated above each line, on ice, for different period of time. Electrophoresis condition same as Figure 3.

Figure 19. The C6ML3-9-SP conjugate protein mediates specific luciferase gene delivery to erbB-2 positive cancer cells

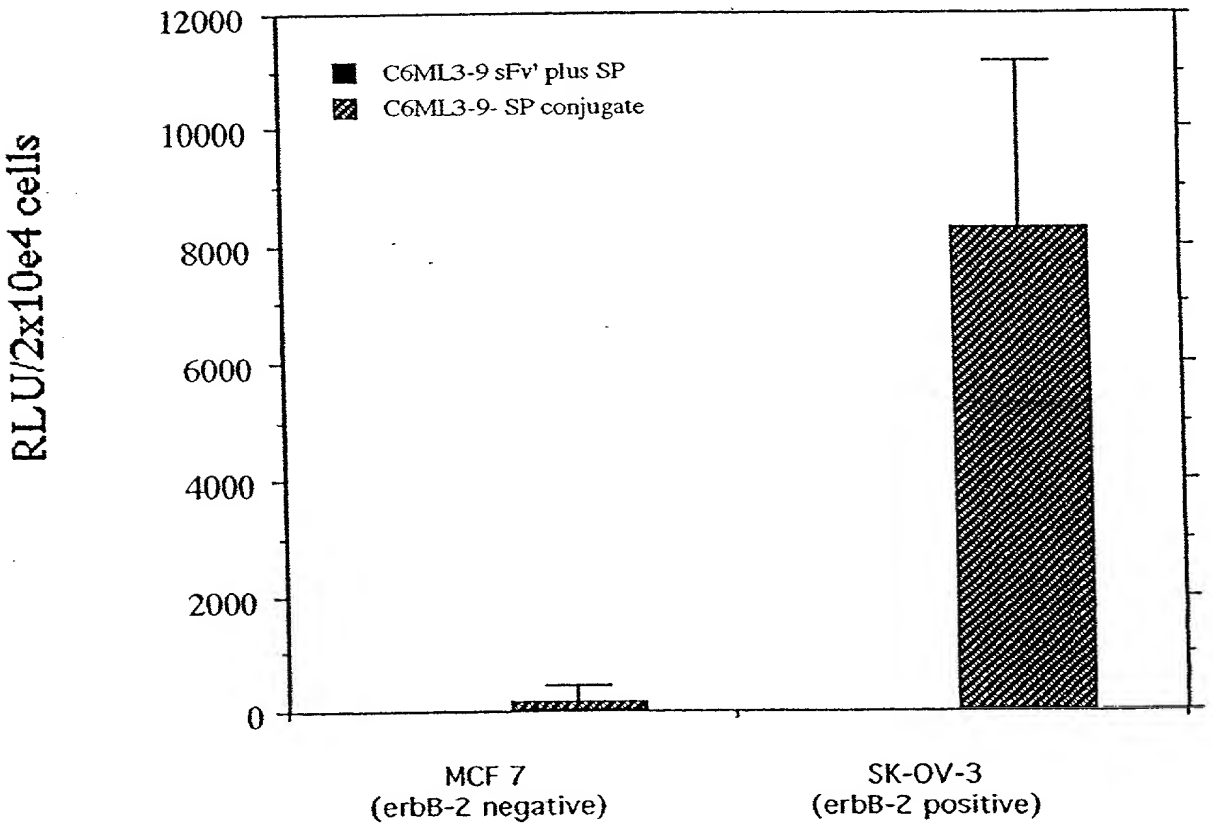


Figure 20. Chloroquine-dependent C6ML3-9-SP-mediated Gene Delivery

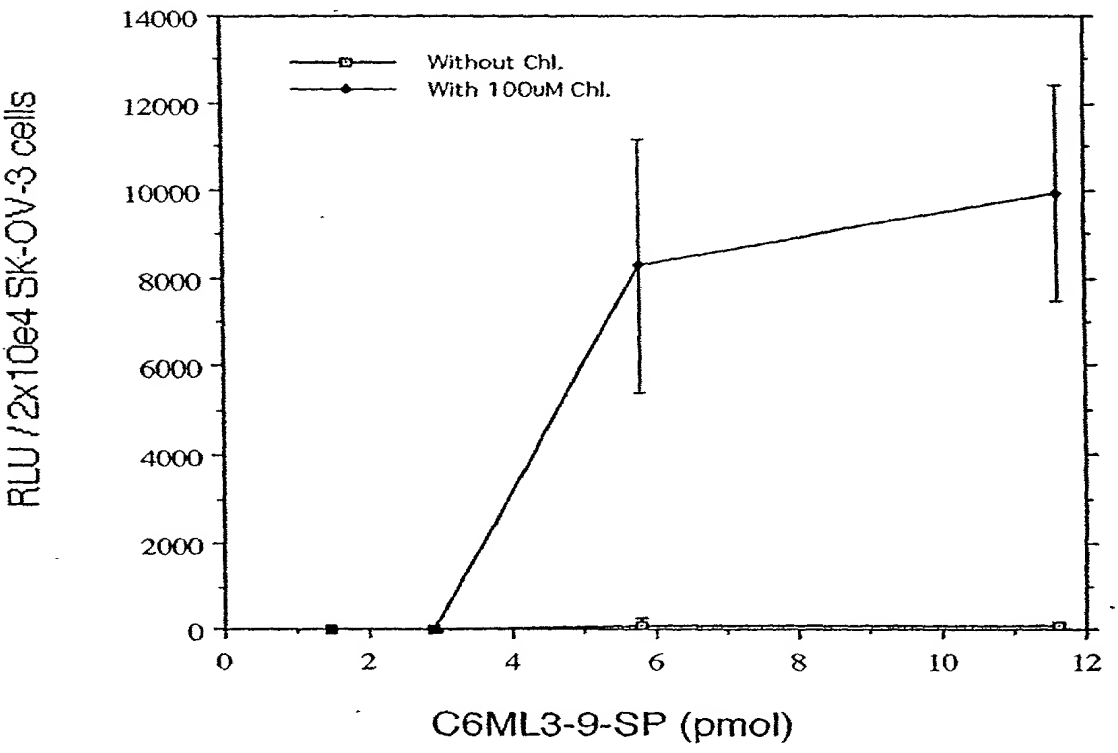


Figure 21. Fluorescent microscopy of C6.5-SP and C6ML3-9 -SP-mediated gene transfer of pGeneGrip Rhodamine/GFP plasmids with SK-OV-3 and MCF-7

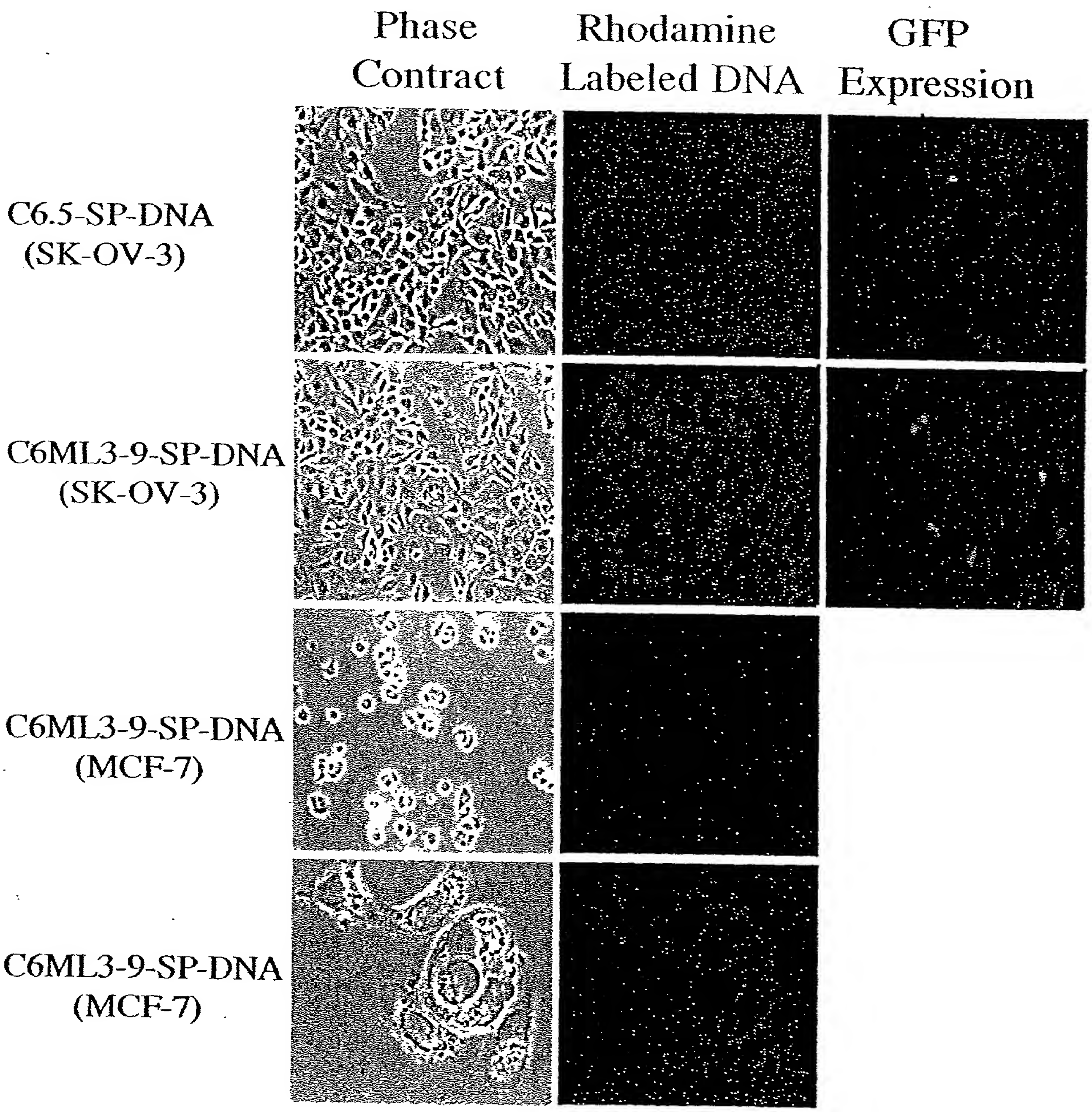


FIGURE 22

THE EFFECT OF
CHLOROQUINE ON 3T3-HER2 TRANSFECTION
MEDIATED BY C6ML3-9sFv'-SALMON PROTAMINE

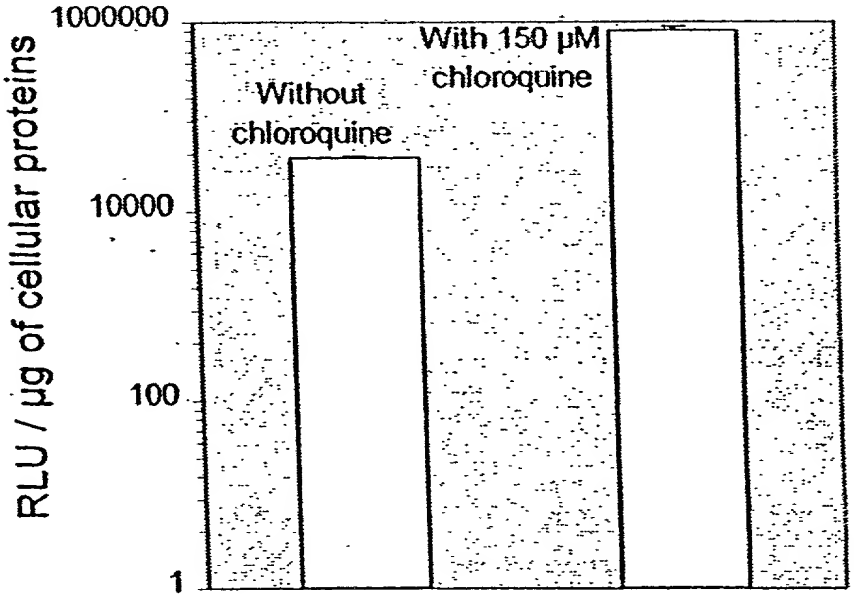


FIGURE 23

THE EFFECT OF CHLOROQUINE ON 3T3-HER2
TRANSFECTION MEDIATED BY C6ML3-9sFv'#2-P1

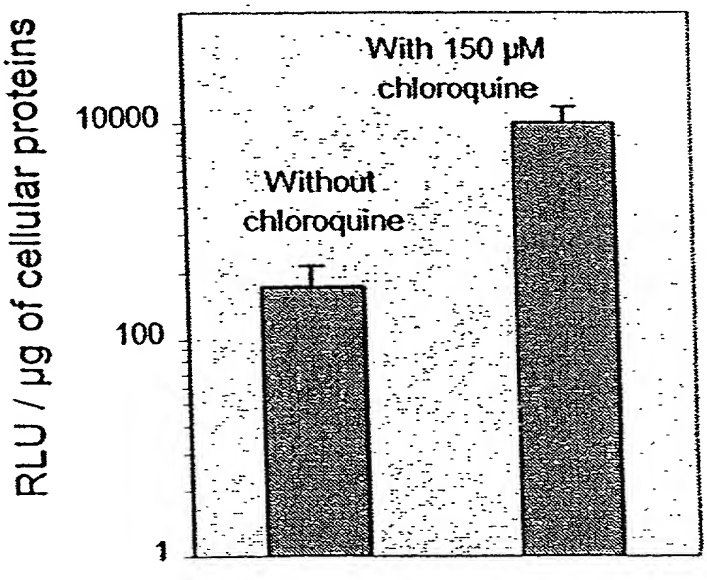


FIGURE 24

THE EFFECT OF CHLOROQUINE ON 3T3-HER2 TRANSFECTION MEDIATED BY C6ML3-9sFv'#2-H1

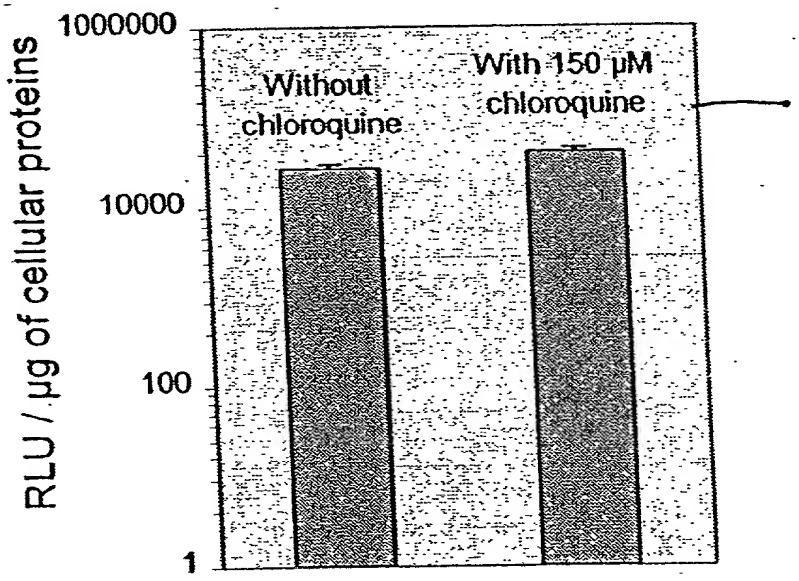


FIGURE 25

THE EFFECT OF C6ML3-9sFv'-H1-pBks ON 3T3-HER2 TRANSFECTION MEDIATED BY C6ML3-9sFv'-H1

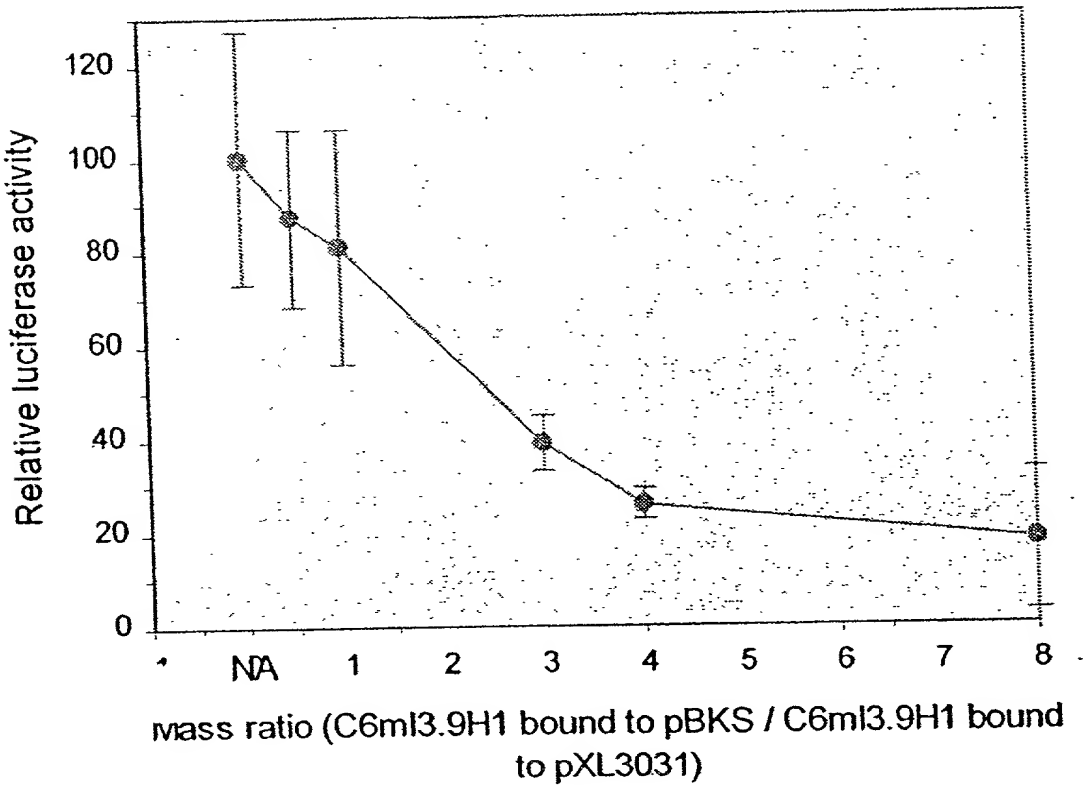


FIGURE 26

THE EFFECT OF THE DNA TO C6ML3-9sFv'-H1 RATIO ON 3T3-HER2 TRANSFECTION EFFICIENCY